CERTIFICATE OF DELIVERY
37 C.F.R. 1.8

I hereby certify that this correspondence is being hand delivered to Examiner Moser with the United States Patent and Trademark Office, Crystal Plaza III, 7th Floor, Washington, DC 20231, on the date below:

Date _____ Signature _____

**PATENT**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application of:
Shuyuan Zhang
Capucine Thwin
Zheng Wu
Toohyon Cho

Serial No.: 09/556,570

Filed: April 24, 2000

For: AN IMPROVED METHOD FOR THE PRODUCTION AND PURIFICATION OF ADENOVIRAL VECTORS

Group Art Unit: 1648

Examiner: M. Mosher

Atty. Dkt. No.: INRP:058--1/SLH

**DECLARATION OF JOSEPH SENESAC UNDER 37 C.F.R. 1.132**

**BOX AF**
Assistant Comissioner of Patents
Washington, D.C. 20231

I, Joseph Senesac, declare that:

1. I am the Manager of Technology Transfer at Introgen Therapeutics, Inc. ("Introgen"), assignee of the above-captioned application. I have been employed at Introgen for six years and have held the Manager of Technology Transfer position for the

last six months. My responsibilities as Manager of Technology Transfer at Introgen have included overseeing adenoviral process development including work with the Adenoviral Reference Material Working Group, which I discuss below. I am a citizen of the United States of America and I reside at 7747 Cambridge # 76, Houston, Texas 77054.

2. Over the last several years, the Food and Drug Administration ("FDA") has been involved in an ongoing evaluation of different adenoviral products and product information from various pharmaceutical companies involved in adenoviral based clinical trials. In January of 2000, the FDA concluded that it was difficult to compare potency, purity and other related parameters of these adenoviral product preparations, mainly due to the lack of conformity with respect to the methods of producing and using adenoviruses. The FDA proposed to those involved in the adenoviral community (*i.e.* regulatory industries, contract testing laboratories, academia, Pharma/Biotech companies, and standard setting organizations (USP, NIST, NIBSC)) that an industry-wide adenoviral standard be developed. The FDA envisioned that standard adenoviral reference material would allow analysis of the safety and efficacy of adenoviral vectors across the product class based on data generated from standardized methods. Furthermore, standard adenoviral reference material would allow the industry to compare, qualify, and validate experiments employing the use of adenoviral vectors.

3. Following the FDA's recommendation, members of the adenoviral community formed what was referred to as the Adenoviral Reference Material Working Group ("ARMWG"). The ARMWG consists of many representatives from institutions including regulatory, academic, pharmaceutical, testing, contract manufacturer, and supplier institutions and has U.S., Canadian, and European representation (Exhibit A).

The ARMWG working in collaboration with the FDA, sought to create an industry-wide standard for adenoviral reference materials that would be accomplished through (1) the donation of a cell bank, (2) the donation of viral starting materials, (3) the production of a viral bank, (4) the production and purification of the adenoviral reference material, (5) the vialing and freezing of reference material, and (6) repository services. To accomplish these goals, the ARMWG, along with the FDA, sent out a bid request. Neither the ARMWG nor the FDA were to fund any activities in connection with the reference material project, and groups were advised that those chosen to perform the above activities would do so voluntarily and without any funding or compensation.

4. Introgen chose to submit Bid Submission Forms, attached as Exhibits B-D, for the above activities because it viewed itself as a leader in the industry of adenoviral products and research. Introgen viewed the opportunity to contribute to creating an industry wide standard for adenoviral reference materials as an important development for the industry as a whole and wanted to participate in its creation.

5. Many groups volunteered and submitted their Bid Submission Forms to the ARMWG and the FDA. The FDA evaluated the proposals and made recommendations to the ARMWG based on the Bid Submission Forms. The ARMWG ultimately made the final selections. As shown in Exhibit E, Introgen was awarded both the (1) production and purification of reference material, and (2) vialing and freezing of reference material.

6. To prepare the adenoviral material that was ultimately selected as the FDA sanctioned Adenoviral Reference Material, the manufacturing staff, under my supervision, employed the adenoviral production and purification process disclosed in the

present specification, as exemplified in FIG. 23 (Exhibit F) with the following non-material changes:

- From the "CUBE" to "HARVEST" step in FIG. 23, the manufacturing staff, under my supervision, did not employ the use of BUFFER A to add the 1% TWEEN-20. Instead, the manufacturing staff added the 1% TWEEN-20 to the media itself.
- From the "HARVEST" to "VIRUS SOLUTION" step in FIG. 23, "CLARIFICATION AND FILTRATION" was performed using a 0.5 μm filtration instead of 0.22 μm filtration.
- From the "VIRUS SOLUTION" to the "CONC. SUP" step in FIG. 23, the manufacturing staff, under my supervision, used 0.5M TRIS instead of 1M NaCl BUFFER A. The results indicated to the right of this step in FIG. 23 remain accurate despite this substitution.
- From the "TREATED SUP" to the "DILUTED VIRUS SOLUTION" step in FIG. 23, the TREATED SUP was not DILUTED WITH WATER TO CONDUCTIVITY = 22-25 mS/cm. The 0.5M TRIS buffer added in the "VIRUS SOLUTION" to "CONC. SUP" acts as a substitute. The results indicated to the right of this step in FIG. 23 remain accurate despite this substitution.

7. Subsequently, the ARMWG requested for further proposals from the industry to perform a variety of tests on the purified adenoviral compositions. One such test was determining the DNA contamination levels of the purified adenoviral compositions. Althea Technologies, Inc. ("Althea") was awarded this activity. Althea is located at 3550 General Atomics Court, Building #2, San Diego, CA 92121.

8. Pursuant to the ARMWG instructions, the ATCC sent the purified adenoviral compositions to Althea for assaying for DNA contamination levels in the compositions. After performing the assays, Althea representatives informed Richard Sublett, Director of Quality Systems at Introgen, that they have never seen DNA contamination levels that low in purified adenoviral compositions. Attached as Exhibit G is the official results of the DNA contamination assays performed by Althea. Specifically, Althea found that the amount of contaminating DNA relative to viral particles ("vp") was 120 pico grams ("pg") per 1 x $10^{12}$ vp in the adenoviral compositions that were purified by the process disclosed in the present application.[1]

9. Althea used a quantitative PCR technique to test for the presence of contaminating DNA relative to virus particles. This technique is designed to detect DNA fragments of greater than 120 base pairs. Thus, DNA fragments less than 120 base pairs were not considered in calculating the amount of contaminating DNA per virus particles.

10. I understand that the present application claims a purified adenovirus composition having a contaminating nucleic acid content of less than about 800 pg and greater than or equal to about 60 pg per about 1.5 x $10^{10}$ to about 2.5 x $10^{10}$ pfu virus. The low end range of contaminating nucleic acid content relative to pfu virus in the present application, *i.e.* 60 pg per about 1.5 x $10^{10}$ pfu to about 2.5 x $10^{10}$ pfu virus, is very comparable with the amount of contaminating DNA found by Althea. For example, 60 pg per about 1.5 x $10^{10}$ pfu virus translates into about 200 pg of contaminating nucleic

[1] Please note that for these results I am referring to Table 2 of Exhibit G. In Table 2, < 3 translates into less than 3 pg HEK 293 DNA in 1 μg total DNA. Also note that 1 x $10^{12}$ vp = 40 μg total DNA. Thus, in 40 μg total DNA there is less than 120 pg HEK 293 DNA per 1 x $10^{12}$ vp.

acid per about 1 x $10^{12}$ vp. Also, 60 pg per about 2.5 x $10^{10}$ pfu virus translates into about 120 pg of contaminating nucleic acid per about 1 x $10^{12}$ vp.

The calculations to convert 60 pg per about 1.5 x $10^{10}$ pfu virus into about 200 pg of contaminating nucleic acid per about 1 x $10^{12}$ vp are as follows:

(a) To convert pfu to vp, the mean vp to pfu ratio in the four adenovrial compositions assayed by Althea was 1 pfu = 20 vp (Exhibit G). Thus, 1.5 x $10^{10}$ pfu converts into 3 x $10^{11}$ vp by the following calculations:

20 vp/1pfu = x vp/1.5 x $10^{10}$ pfu where x = 3 x $10^{11}$.

(b) 60 pg/3 x $10^{11}$ vp converts into 200 pg/1 x $10^{12}$ vp by the following calculations:

60 pg/3 x $10^{11}$ vp = x pg/1 x $10^{12}$ vp where x = 200 pg.

The calculations to convert 60 pg per about 2.5 x $10^{10}$ pfu virus into about 120 pg of contaminating nucleic acid per about 1 x $10^{12}$ vp are as follows:

(a) 2.5 x $10^{10}$ pfu converts into 5 x $10^{11}$ vp by the following calculations:

20 vp/1pfu = x vp/2.5 x $10^{10}$ pfu where x = 5 x $10^{11}$.

(b) 60 pg/5 x $10^{11}$ vp converts into 120 pg/1 x $10^{12}$ vp by the following calculations:

60 pg/5 x $10^{11}$ vp = x pg/1 x $10^{12}$ vp where x = 120 pg.

11. I declare that all statements made herein of my own knowledge are true, and that all statements of my own belief are believed to be true, and further that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under § 1001 of title 18 of the United States Code, and that

such willful false statements may jeopardize the validity of this patent, and any reexamination certificate issuing thereon.

30 January 2002
Date

Joseph Senesac
Joseph Senesac

# EXHIBIT A

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| 6 | ~~Pennathur-Das, Rukmini~~ (P-D Rukminihas has dropped out due to purchase of Calydon by Cell Genesys) | ~~Calydon~~ | ~~Pharma~~ | ~~Director~~ | ~~rdas@calydon.com~~ | ~~408-752-8740~~ | ~~408-734-1714~~ | ~~1324 Chesapeake Terrace, Sunnyvale, CA 94089~~ | |
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| 9 | Borellini, Flavia | Cell Genesys | Pharma | Director, Assay Dev/QC | flaviab@cellgenesys.com | 650-425-4459 | 650-358-0440 | 342 Lakside Dr, Foster City, CA 94404 | |
| | Nesbit, Heike | Cell Genesys | Pharma | | | | | | |
| 10 | Couture, Larry | City of Hope Nat'l Med. Ctr. | Academic | Vice President | lcouture@coh.org | 626-256-8728 | 626-256-8730 | Beckman Research Instit. Ctr. For Applied Biomed. & Genetics 1500 E. Duarte Rd. Duarte, CA 91010 | |
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| 16 | Butman, Bryan | GenVec, Inc. | Pharma | Director | bbutman@genvec.com | 240-632-0740 | 240-632-0735 | 65 West Watkins Mill Rd., Gaithersburg, MD 20878 | |
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| 20 | Cornavaca, Eric | Invitrogen Corp. | Supplier | Director, Business Development | eric.cornavaca@invitrogen.com | 718-774-6660 | 716-774-6760 | Gibco Cell Culture Products, Invitrogen Corp., 3175 Staley Rd., Grand | |
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| | Vellekamp, Gary | Schering Plough Research Institute | Pharma | Sr Principal Scientist | gary.vellekamp@spcorp.com | 908-820-6181 | 908-820-6785 | 1101 Morris Ave., Union, NJ 07083 | |
| 28 | Printz, Marie | Selective Genetics | Pharma | Assay Development | mprintz@selectivegenetics.com | 858-625-0100 | 858-625-0222 | 11035 Roselle St., San Diego, CA 92121 | |
| 29 | Koehl, Michel | Transgene | Pharma | Head, Proc Developmt | koehl@transgene.fr | +33 0 3 88 27 9110 | +33 03 88 22 5807 | 11, Rue de Molsheim, 67082 Strasbourg Cedex, France | Edwige Bonfils |
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| 30 | Croyle, Maria | Univ. of Texas - Austin | Academic | Assist. Prof. Pharmaceutics | macroyle@mail.utexas.edu | 512-471-1972 | 512-471-7474 | College of Pharmacy PHR 4.214D Austin, TX 78712 | |
| 31 | Carson, Keith | Williamsburg BioProcessing Fdtn | Academic | | kcarson@wilbio.com | 757-423-8823 | 757-423-2065 | 4015 Killiam Ave., Norfolk, VA 23508 | Manisha Trivedi |

# EXHIBIT B

# Adenoviral Reference Material Working Group
# Bid Submission Form
# Viral Bank Production and Testing Donation
# RFP 4.0

Item for Submission

Production of an Ad5 Wild-type Virus Bank in lysate form to support production of 10 lots of adenovirus reference material. The bidder will also provide a Certificate of Analysis summarizing characterization as called for below. The delivered virus bank will need to at a minimum be 5 x $10^{13}$ total particles in at least 20 aliquots.

General Requirements for Bidding

The Virus Bank will need to be produced under well-documented conditions (not necessarily equivalent to CGMP). Virus stock and cells used for bank production will be supplied from other bid activities in this process. Indicate your minimum requirements / concerns for acceptance of these materials (cell bank vials and viral seed material). The bid should indicate the amount of time required from receipt of the cell bank vials and viral material for the production and release of the virus bank.

The institution will need to provide a brief statement describing the proposed method for production of this bank, including proposed vial configuration. The institution should include their proposed specifications, including information on the proposed test methods, and a proposed Certificate of Analysis addressing the following points:

?? Identity
?? Particle concentration
?? Functional activity (such as infectivity)
?? Sterility (USP or 21CFR610.12)
?? Mycoplasma
?? *In vitro* adventitious viral agents, or equivalent
?? *In vivo* adventitious viral agents
?? Human pathogens:
    EBV
    HIV 1& 2
    HTLV 1& 2
    HBV
    HCV
    CMV
    Parvo B19
    AAV
?? Bovine virus – Certificate of Analysis where applicable (raw material or final vial test) (per 9CFR113.47)

?? Porcine parvovirus – Certificate of Analysis where applicable (raw material trypsin or final vial test)

The proposal should include details of the proposed method/container for shipping to ensure integrity of the viral bank vials upon arrival at the production facility for the purified reference material.

Documentation Requirements
A description of the method proposed for production of the virus bank.

A proposed Certificate of Analysis should be provided addressing the characterization described above along with the proposed specifications, and information on the proposed test methods,

Proposed details related to shipping of virus bank vials to production facility.

In addition to these specific documentation requirements, each institution bidding should include a brief statement describing their experience and capacity to perform such activity and a description of the facility in which the work will be performed. The facility description should address procedures to ensure segregation during viral bank production.

Please complete the following fields:

*Contact Information – RFP 4.0*

| | |
|---|---|
| **Contact Individual:** | Joe Senesac |
| **Institution:** | Introgen Therapeutics, Inc. |
| **Address:** | 2250 Holcombe Blvd.<br>Houston, Texas 77030 |
| **Phone Number:** | (713) 610-4020 |
| **Email Address:** | j.senesac@introgen.com |

*Viral Bank Production and Testing Donation Information – RFP 4.0*

Please indicate if your institution is also submitting proposals for the other activities:

- ☐ Donation of Cell Bank
- ☐ Donation of Ad5 Wild-type Virus
- ☒ Ad5 Wild-type Purified, Formulated Bulk Production
- ☐ Donation of Repository Services
- ☒ Vialing of Ad5 Wild-type Reference Material
- ☐ Donation of Supplies/Other Services

*Please attach*:

Proposed Certificate of Analysis, specifications, and summary of test methods
Description of production of viral bank including vial configuration
Facility information
Information on shipping

Submit this completed form and all attached information for receipt **by February 28, 2001** to the address below. Electronic submissions are encouraged. Final decisions will be communicated by or about March 31, 2001. Please note that all information submitted will be publicly available. Please do not mark any information confidential, as we cannot honor that request. Please include an estimated cost and market value of all goods and services donated.

**Williamsburg BioProcessing Foundation**
**Attn: Adenovirus Reference Material Working Group**
**4015 Killam Avenue**
**Norfolk, VA 23508**

**PH: 757-423-8823**
**FAX: 757-423-2065**

**EMAIL: advector@wilbio.com**

## A. Capability Statement

Introgen Therapeutics, Inc. (Company) is engaged in the manufacture, research and clinical development of viral vector-based gene therapies for cancer treatment. The Company utilizes a 12,000 square foot manufacturing facility at 2252 Holcombe Boulevard in Houston, TX for the production of viral vectors for non-clinical and clinical studies. This facility is fully commissioned and has been qualified via the production of three lots of an adenoviral vector, RPR/INGN 201, containing a functional copy of the human *p53* gene Ad5CMV-*p53*.

Introgen has produced over 30 clinical batches of various materials for Phase I to Phase III clinical studies. The facilities include class 100,000 down to Class 1,000 cleanrooms which provide two separate manufacturing suites and appropriate environments for Cell/Viral Culture, Purification, and Finishing activities. Key members of the manufacturing team have worked together at Introgen for greater than 5 years. Introgen also has fully staffed Quality Assurance and Quality Control departments for testing and oversight of production.

## B. Production Requirements

Cell Bank Vials

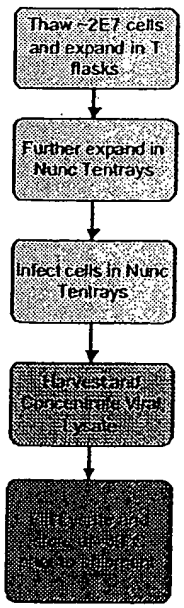
Production will require a minimum of 4E7 total viable adherent cells in multiple vials. Vials are to be shipped to Introgen in the vapor phase of Liquid Nitrogen. Required documentation will include the completed Certificate of Analysis as specified in RFP 1.0, and a description of cell culture techniques specific to the cell line. Cell culture media is to be defined as to vendor and catalog number.

Viral Material Vials

Production will require a minimum of 1E12 viral particles, as stated in RFP 2.0, to be shipped to Introgen on dry ice. Required documentation will include the characterization and instructional information also as specified in RFP 2.0.

## C. Viral Bank Production

Production Flowchart:



Process Description

The adherent cell line will be expanded first using T150 flasks, then using Nunc Tentray Cell factories. Flasks will be incubated in a $CO_2$ and humidity controlled incubator. All cell and viral processing will take place in a class 100 biosafety cabinet (BSC) located in a class 10,000 cleanroom.

General information

The production will take place on a strict campaign basis, with the rooms thoroughly cleaned and disinfected prior to and at the end of production. Production personnel will be limited to working with a single construct each working day. Production materials are sterile single use disposable. Equipment is thoroughly decontaminated and cleaned prior to being sterilized or sanitized for use.

Cell Culture

Cells are thawed at 37?C prior to placement in a BSC and transfer to sterile disposable culture flasks. The adherent cells will be expanded in a timeframe based on the directions of the cell bank donor. Expansion will start in T flasks and will progress through the use of disposable Nunc Cell Factories until a cell mass is reached that is sufficient for infection. Infection is accomplished by draining the media in the Nunc Cell Factories, then replacing with fresh media containing the viral material at the appropriate concentration.

Harvest and Processing

The harvested material will be filter clarified prior to concentration. The formulated bulk will be 0.2 um filtered and vialed prior to freezing at ?-60?C.

## D. Production Schedule

Total production time will be dependent upon the characteristics of the cell line and viral material used for production. An estimate of 3 weeks total time can be made from previous Introgen experience with these activities and the scale of the virus bank to be made. Release testing will require approximately an additional twelve weeks.

## E. Formulation

Crude concentrated harvest.

## F. Container Closure and Shipment

The viral bank material vials will be stored at ? -60?C prior to shipment to the reference material production site. The vials will be shipped to the production site on dry ice in a thermal shipping container. A calibrated temperature trace device can be included with the shipment if desired.

The glass vials used are contract supplied to Introgen Therapeutics, Inc. as sterile stoppered vials. The stoppered vials are aseptically packaged in stainless steel racks that are individually triple wrapped for cleanroom use.

Container closure details are as follows:

Vial - 3mL, 13MM Serum/Lyophilization Vial, Flint glass

Stopper – 13MM Grey Butyl Stopper

Crimp- Flip-off Button Crimp

## G. Certificate of Analysis and Specifications

See attached proposed Certificate of Analysis containing specifications.

## H. Documentation

A detailed production report will be submitted upon completion of testing. The report will document the production methods and will be included in lieu of actual production records.

**Introgen Therapeutics, Inc.**
**Testing Summary for Serotype 5 Reference Material**
**Master Virus Bank**
**Lot #**

Manufactured By: Introgen Therapeutics, Inc.
Adenovirus Serotype 5 Reference Material
Date of Manufacture:
Virus Stock:
Cell Bank:

Lot Number
Nominal Fill Volume:
Number of Containers:
Store at -60°C or below

| Test | Sponsor | Specification | Result |
|---|---|---|---|
| **Supernatant Harvest** | | | |
| Mycoplasma Cultivable and Non Cultivable | MDS Panlabs | Negative | |
| Bioburden | Introgen | Report Value | |
| *In Vivo* Adventitious Virus | TBD* | Negative | |
| Adeno-Associated Virus (PCR) | TBD* | Negative | |
| B19 (Parvo) | TBD* | Negative | |
| CMV | TBD* | Negative | |
| EBV | TBD* | Negative | |
| Hep B | TBD* | Negative | |
| HIV1 and HIV2 | TBD* | Negative | |
| HTLV I&II | TBD* | Negative | |
| Hep C | TBD* | Negative | |
| **Master Virus Bank** | | | |
| Sterility | MDS Panlabs | Sterile | |
| Bacterial Endotoxin (LAL) | Introgen | Report Value | |
| Plaque Titration of Adenovirus Vector | Introgen | Report Value | |

**Introgen Therapeutics, Inc.**
**Testing Summary for Serotype 5 Reference Material**
**Master Virus Bank**
**Lot #**

I certify that the above information has been accurately transcribed.

By: _____ _____
Quality Assurance Representative Date

* Testing will be performed at a CGMP facility but Introgen has not yet determined which test site will be used.

# EXHIBIT C

# Adenoviral Reference Material Working Group
# Bid Submission Form – Purified Formulated Bulk Virus Reference Material Production and Release Testing Donation
# RFP 5.0

Item for Submission

Production and release testing of an Ad5 Wild-type Virus Bulk Reference Material in final formulation ready to be vialed. The bidder will also provide a Certificate of Analysis summarizing characterization as called for below. The intended lot size for the reference material is to enough bulk for vialing of 4500 to 5000 x 0.5 mL vials at 2 to 5 x $10^{11}$ particles/mL, or equivalent to approximately 1.3 x $10^{15}$ total particles after release. The bulk material should be delivered in at least 4 aliquots.

General Requirements for Bidding

Reference material will need to be produced under conditions equivalent to CGMP. Virus bank and cell bank vials used for reference material production will be supplied from other bid activities in this process. Indicate your minimum requirements / concerns for acceptance of these materials (cell bank vials and viral bank vials) if not addressed by characterization called for RFP 4.0. The bid should indicate the amount of time required from receipt of the cell bank vials and viral bank vials for the production and release of the purified, formulated bulk reference material.

The institution will need to provide a brief statement describing the proposed method for production and purification of the reference material bulk, including proposed container configuration. The institution should include their proposed specifications, including information on the proposed test methods, and proposed Certificate of Analysis addressing the following points:

?? Identity
?? Purity
?? Provisional Particle concentration
?? Functional activity (such as infectivity)
?? Sterility (USP or 21CFR610.12)
?? Mycoplasma
?? Endotoxin
?? *In vitro* adventitious viral agents or equivalent
?? AAV

And where applicable

?? Bovine virus – Certificate of Analysis (raw material or final vial test) (per 9CFR113.47)
?? Porcine parvovirus – Certificate of Analysis (raw material trypsin or final vial test)

The proposal should include details of the proposed method/container for shipping to ensure integrity of the bulk material upon arrival at the vialing facility. The proposal should also include instructions regarding how the material is to be dispensed. Examples are "the material will be shipped at 2-8?C and should be dispensed before freezing," or, "the material will be shipped frozen; it should be thawed under the following conditions (supplied) and then should be dispensed and then frozen."

In addition to these specific documentation requirements, each institution should include a brief statement describing their experience and capacity to perform this activity and a description of the facility in which the work will be performed. The facility description should address procedures to ensure segregation during viral bulk reference material production and purification.

It is expected that the final documentation package made available with the formulated bulk would include copies of the completed batch records used for production.

Documentation Requirements

Documentation should include detailed information on a proposed final formulation, which should not be solely PBS (phosphate buffered saline)-based nor should it contain protein. This information should include supporting data indicating the formulation's ability to provide stability for storage of Adenovirus at $\leq$-55?C. The formulation information should also indicate compatibility with biological and chemical characterization methods. The working group will determine the final formulation used.

The bid should include a description of the proposed cell and viral culture, harvest, and purification methods along with the proposed specifications, test methods, and proposed Certificate of Analysis.

The proposal should include details of the proposed method/container for shipping the formulated bulk reference material to the vialing facility.

Please complete the following fields:

*Contact Information – RFP 5.0*

| | |
|---|---|
| **Contact Individual:** | Joe Senesac |
| **Institution:** | Introgen Therapeutics, Inc. |
| **Address:** | 2250 Holcombe Blvd.<br>Houston, Texas 77030 |
| **Phone Number:** | (713) 610-4020 |
| **Email Address:** | j.senesac@introgen.com |

*Purified Formulated Bulk Virus Reference Material Production and Release Testing Donation – RFP 5.0*

Indicate Propagation Method: ☐ Suspension ☒ Adherent

Please indicate if your institution is also submitting proposals for the other activities:

- ☐ Donation of Cell Bank
- ☐ Donation of Ad5 Wild-type Virus
- ☒ Ad5 Wild-type Virus Bank Production
- ☐ Donation of Repository Services
- ☒ Vialing of Ad5 Wild-type Reference Material
- ☐ Donation of Supplies/Other Services

*Please attach:*

Proposed Certificate of Analysis, specifications, and test methods
Proposed method for production and purification
Proposed Formulation Information
Institution Capability Statement
Information on shipping

Submit this completed form and all attached information for receipt **by February 28, 2001** to the address below. Electronic submissions are encouraged. Final decisions will be communicated by or about March 31, 2001. Please note that all information submitted will be publicly available. Please do not mark any information confidential, as we cannot honor that request. Please also include an estimate of cost and market value of donated goods and services.

**Williamsburg BioProcessing Foundation**
**Attn: Adenovirus Reference Material Working Group**
**4015 Killam Avenue**
**Norfolk, VA 23508**

**PH: 757-423-8823**
**FAX: 757-423-2065**

**EMAIL: advector@wilbio.com**

## A. Capability Statement

Introgen Therapeutics, Inc. (Company) is engaged in the manufacture, research and clinical development of viral vector-based gene therapies for cancer treatment. The Company utilizes a 12,000 square foot manufacturing facility at 2252 Holcombe Boulevard in Houston, TX for the production of viral vectors for non-clinical and clinical studies. This facility is fully commissioned and has been qualified via the production of three lots of an adenoviral vector, RPR/INGN 201, containing a functional copy of the human *p53* gene Ad5CMV-*p53*.

Introgen has produced over 30 clinical batches of various materials for Phase I to Phase III clinical studies. The facilities include class 100,000 down to Class 1,000 cleanrooms which provide two separate manufacturing suites and appropriate environments for Cell/Viral Culture, Purification, and Finishing activities. Key members of the manufacturing team have worked together at Introgen for greater than 5 years. Introgen also has fully staffed Quality Assurance and Quality Control departments for testing and oversight of production.

## B. Production Requirements

Cell Bank Vials

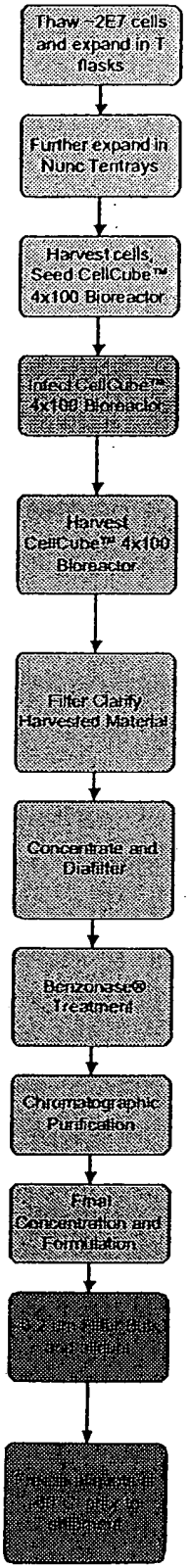
Production will require a minimum of 4E7 total viable adherent cells in multiple vials. Vials should be shipped to Introgen in the vapor phase of Liquid Nitrogen. Required documentation will include the completed Certificate of Analysis as specified in RFP 1.0, and a description of cell culture techniques specific to the cell line. Cell culture media is to be defined as to vendor and catalog number.

Virus Bank Vials

Production will require a minimum of 4E12 viral particles to be shipped to Introgen on dry ice. Required documentation will include the completed Certificate of Analysis as specified in RFP 4.0.

## C. Formulated Bulk Virus Production

Production Flowchart


Thaw ~2E7 cells and expand in T flasks
Further expand in Nunc Tentrays
Harvest cells, Seed CellCube™ 4x100 Bioreactor
Infect CellCube™ 4x100 Bioreactor
Harvest CellCube™ 4x100 Bioreactor
Filter Clarify Harvested Material
Concentrate and Diafilter
Benzonase® Treatment
Chromatographic Purification
Final Concentration and Formulation

General information

Cell culture operations take place in a class 10,000 cleanroom equipped with a class 100 biosafety cabinet (BSC). Bioreactor culture and downstream processing will take place in a class 100,000 cleanroom equipped with class 100 BSCs and portable ULPA laminar air stations. All equipment and HEPA filters have current calibration or certification. The production in the facility is campaigned, with no overlap between lots of material produced in a suite, and with the rooms thoroughly cleaned and disinfected at the end of production and prior to beginning the next batch. Production personnel are limited to working with a single construct per working day. Production materials are sterile single-use disposables wherever possible. Equipment that is not disposable is thoroughly decontaminated and cleaned prior to being sterilized or sanitized for use.

Cell/Viral Culture

Cells are thawed at 37?C prior to placement in a BSC and transfer to sterile disposable culture flasks. The adherent cells will be expanded in a timeframe based on the directions of the cell bank donor. Expansion will start in T flasks and will progress through the use of disposable Nunc Cell Factories until a cell mass is reached that is sufficient to seed the CellCube™ 4x100 Bioreactor. It is anticipated that a single CellCube™ 4x100 Bioreactor run will produce sufficient product for the scope of this project. The CellCube™ 4x100 Bioreactor and tubing set are disposable. The associated oxygenator and probes are reusable.

The cells will be allowed to expand for a predetermined number of days prior to infection. To infect the CellCube™ 4x100 Bioreactor, the virus bank is injected via an access port. The bioreactor is incubated for a predetermined number of days prior to harvest.

Harvest and Downstream Processing

The harvested material will be filter clarified prior to concentration and diafiltration. Benzonase® treatment to reduce residual host cell nucleic acids will also be performed prior to purification.

Column chromatography will be used to purify the material, which will then be further concentrated and formulated into the designated formulation. The final formulation will be prepared with specified USP chemicals and USP water for injection.

The formulated bulk will be 0.2 um filtered and aliquoted prior to freezing at ? -60?C.

## D. Production Schedule

After receipt of the cell bank vials a three-week trial period would be necessary to assess cell growth characteristics and to develop documentation. The production

of the formulated Bulk product would require approximately one month. Release testing will require a period of 12 weeks.

## E. Final Formulation and Container/Shipment

The final formulation is 20mM TRIS, pH 8.0, 2.5% Glycerol, 25mM NaCl, which has been donated by Selective Genetics. This formulation has demonstrated good stability characteristics as detailed in the attached Formulation file.

The Bulk material will be sterile filtered and aliquoted into sterile 50mL polypropylene tubes for shipment. An estimated 50-60 tubes will be filled and appropriately labeled prior to freezing at ? -60?C.

Shipment of the aliquots will be on dry ice with the addition of a calibrated temperature trace device to document shipping conditions. The shipping container will be an EnduroTherm® insulated container.

The material should be thawed at room temperature prior to sterile filtration and product fill.

## F. Certificate of Analysis and Specifications

See attached proposed Certificate of Analysis containing specifications.

## G. Documentation

A detailed production report will be submitted upon completion of testing. The report will document the production methods and will include the completed Certificate of Analysis. This will be made available in lieu of completed production batch records.

**Introgen Therapeutics, Inc.**
**Certificate of Analysis for Formulated Bulk Adenovirus Serotype 5**
**Reference Material**
**Lot #**

Manufactured By: Introgen Therapeutics, Inc.
Adenovirus Serotype 5 Reference Material
Date of Manufacture:
Virus Bank:

Lot Number:
Volume
Store at -60°C or below
Cell Bank:

| Test | Sponsor | Specification | Result |
|---|---|---|---|
| **Pre-Infected Cells** | | | |
| In-vitro Adventitious Virus | TBD* | No evidence of adventitious viral agents | |
| **Crude Cell Lysate** | | | |
| Mycoplasma EP & PTC 1993 | MDS Panlabs | Negative for presence of Mycoplasma | |
| Bioburden | TBD* | Report Value | |
| Adeno-Associated Virus (PCR) | TBD* | Negative for Adeno-associated Virus | |
| **Purified Bulk Product (Postfilter)** | | | |
| Sterility USP & EP | MDS Panlabs | Sterile | |
| Bacterial Endotoxins Test | Introgen | Report Value | |
| Plaque Titration of Adenovirus Vector | Introgen | Report Value | |
| Virus Particle Enumeration by $OD_{260}$ | Introgen | 2E11 – 5E11 vp/mL | |
| Purity by HPLC Ion Exchange | Introgen | ? 98% Purity | |
| Bovine Serum Albumin (ELISA) | Introgen | Report Value | |
| huDNA | TBD* | Report Value | |
| pH | Introgen | 7.6 – 8.4 | |

I certify that the above information has been accurately transcribed.

By: _____ _____
Quality Assurance Representative Date

* Testing will be performed at a CGMP facility but Introgen has not yet determined which test site will be used.

As part of RFP 5.0, Mark D'Andrea (Senior Director, Process Development), representing Selective Genetics, Inc. (11035 Roselle St., San Diego, CA, 92121), respectfully submits an aqueous, Tris-buffered formulation containing glycerol and NaCl for use as the final formulation of the purified reference standard material. The following details the composition of the formulation and provides stability data demonstrating the usefulness of this specific formulation.

## 1. Summary

The stability of first generation (E1, E3 deleted) recombinant adenovirus type 5 encoding the PDGF-B (Platelet-derived growth factor B) gene, was studied by subjecting formulated, purified virus to freeze-thaw and temperature stress at various adenovirus concentrations from $1.7x10^{10}$–$1.7x10^{12}$ PN/ml (adenoviral particle number per milliliter). The GTS formulation (2.5% glycerol v/v, 20 mM Tris pH 8, 25 mM NaCl) provided good stability for adenovirus at all particle concentrations tested from $1.7x10^{10}$ to $1.7x10^{12}$/ml, whether stored in plastic or glass vials.

At temperatures of 2-8?C and higher, virus stability was dependent on particle concentration. At the highest concentration ($1.7x10^{12}$ PN/ml), a 15% and 65% decrease in particle recovery was observed at 3 and 6 months, respectively. At concentrations equal to or lower than $1.7x10^{11}$ PN/ml, GTS formulated adenovirus has remained stable for at least 6 months when stored at 2-8°C in cryovials. Concentrations = $5.1x10^{11}$ PN/ml contained in glass vials have shown stability at 2-8°C for at least 3 months.

## 2. Stability indicating methods

Analytical methods including anion exchange HPLC (AE-HPLC), Laser light scattering (LLS) and a bioactivity (ELISA) assay were developed as stability indicating methods. AE-HPLC and functional gene expression assays were used to examine the structural integrity and activity of adenovirus. Laser light scattering (LLS) was used to monitor viral particle aggregation, an early event in purified virus degradation.

*AE-HPLC Particle Determination*
Particle concentrations were analyzed by AE-HPLC utilizing a Resource Q column attached to an HP1050 HPLC. Using this method, intact adenoviral particles are quantified at a detection limit of ca. $2x10^{10}$ PN/ml, and a limit of quantitation of ca. $3.8x10^{10}$ PN/ml. Particle concentrations are calculated by comparing average peak areas to a calibration curve prepared from the analysis of a reference standard.

*Laser Light Scattering (LLS)*
The average virus particle size is determined by LLS using a Zetasizer 5000 (Malvern Instruments, England). The average hydrodynamic diameter ($Z_{ave}$, nm) is measured with an argon-ion laser operating at 488 nm, 15 to 50 milliwatts, and a 90? angle. The hydrodynamic diameter ($Z_{ave}$) describes the apparent size of the particle as it exists in the solution, and is reported as the average size of the entire population. Adenoviral vectors have an apparent size range of 80-120 nm. The homogeneity (or polydispersity) of the population is reported using a polydispersity index which indicates the particle size

distribution within the population. As the polydispersity index approaches 1.0, the sample is more likely to contain multiple populations of varying $Z_{ave}$. A monodisperse distribution will have a polydispersity index of <0.2.

*AdPDGF-B Transduction/PDGF-BB Expression*
To confirm PDGF-BB expression, 293 cells are transduced and cell lysate supernatant subjected to ELISA analysis. ELISA plates (96-well) are coated with the human PDGF receptor ?/Fc chimera which captures the PDGF-BB dimer. Recombinant human PDGF-BB protein is used as a positive control. Activity is quantitated using the concentration of AdPDGF-B which produces 50% maximal response (effective concentration-50; $EC_{50}$) in PN/cell, or nanograms PDGF-BB at a specific PN/cell ratio.

**3. Experimental**
*Lot A:*
Aliquots of Lot A were filled into sterile 1.8 ml cryovials at 3 different viral particle concentrations: $1.7x10^{12}$ PN/ml, $1.7x10^{11}$ PN/ml and $1.7x10^{10}$ PN/ml. For freeze-thaw testing, high and mid-concentration samples were frozen in an ethanol-dry ice bath for 5 minutes and thawed at room temperature (20-25?C) for 15-20 min. For long-term temperature stability studies, vials were stored in temperature controlled environments.

*Lot B:*
Aliquots of Lot B were filled into sterile 3 ml, 13 mm serum/lyophilization vials, sealed with 13 mm grey butyl stoppers and crimped with flip-off button crimps. Lot B was formulated at high, middle and low particle concentrations: $5.1x10^{11}$ PN/ml, $1.7x10^{11}$ PN/ml and $5.1x10^{10}$ PN/ml, respectively. For freeze-thaw studies, each vial was frozen in a -20?C non-cycling freezer for ?3 hours and thawed at 20-25?C for 30-60 min. For long-term temperature stability studies, vials were stored in temperature controlled environments.

**4. Results:**
*Freeze-Thaw Stability*
As shown in Tables 1 and 2, the particle size and polydispersity of samples for both Lots A and B remained unchanged following 1, 3, and 5 freeze-thaw cycles. The low particle concentration samples of Lot A and Lot B precluded LLS analysis. Similarly, AE-HPLC results indicated quantitative recovery of intact particles (Figures 1A and 2A). Lastly, no changes in PDGF-BB expression, as compared to a fresh control (0 F-T), were demonstrated by AdPDGF-B transduction/PDGF-BB ELISA analysis (Figure 1B, and 2B). These data indicate that both AdPDGF-B Lots remain stable for at least 5 freeze-thaw cycles whether stored in plastic or glass vials.

**Table 1. Particle Size and Polydispersity of Lot A Subjected to Freeze-Thaw Stress**

| # Freeze-thaws cycles | 0 | | 1 | | 3 | | 5 | |
|---|---|---|---|---|---|---|---|---|
| | $Z_{ave}$ | Poly-dispersity | $Z_{ave}$ | Poly-dispersity | $Z_{ave}$ | Poly-dispersity | $Z_{ave}$ | Poly-dispersity |
| $1.7x10^{12}$ PN/mL | 106 | 0.06 | 102 | 0.12 | 101 | 0.12 | 101 | 0.11 |
| $1.7x10^{11}$ PN/mL | 100 | 0.10 | 102 | 0.10 | 100 | 0.08 | 101 | 0.10 |

**Figure 1. AE-HPLC and PDGF-BB Expression Analysis of Lot A Subjected to Freeze-Thaw Stress**

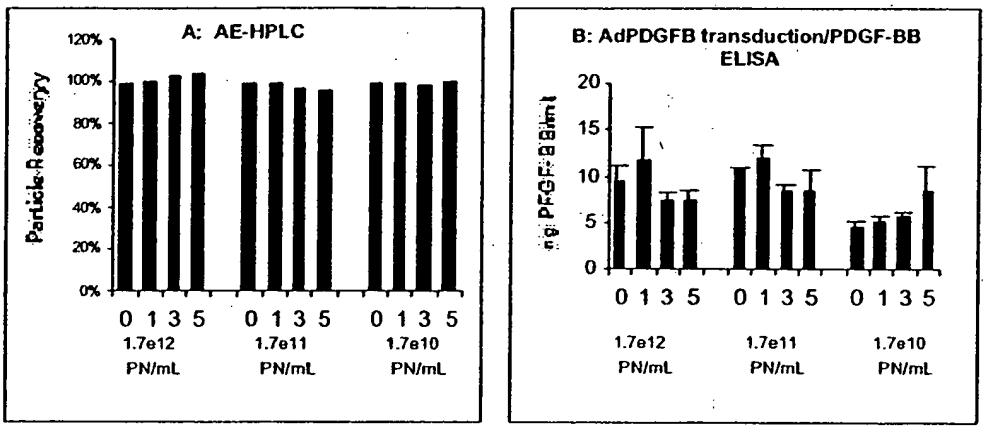
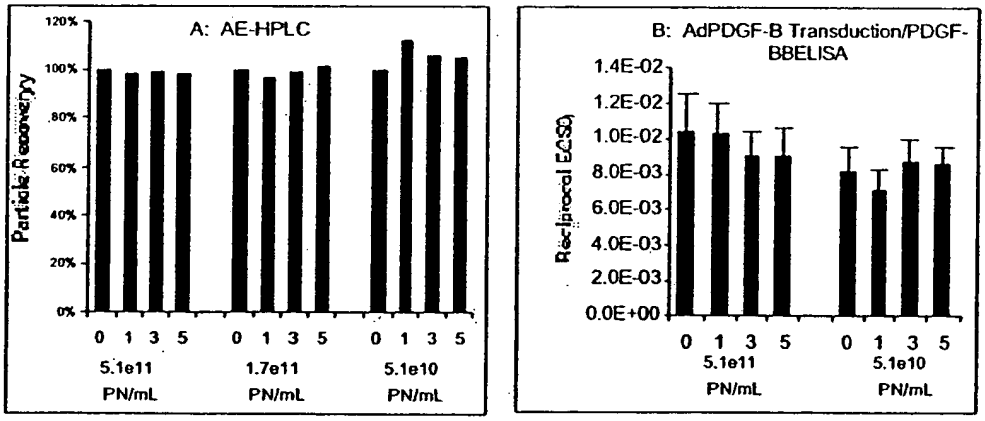



**Table 2. Particle Size and Polydispersity of Lot B Subjected to Freeze-Thaw Stress**

| # Freeze thaw cycles: | 0 | | 1 | | 3 | | 5 | |
|---|---|---|---|---|---|---|---|---|
| | $Z_{ave}$ | Poly-dispersity | $Z_{ave}$ | Poly-dispersity | $Z_{ave}$ | Poly-dispersity | $Z_{ave}$ | Poly-dispersity |
| $5.1x10^{11}$ PN/mL | 104 | 0.06 | 103 | 0.06 | 104 | 0.04 | 103 | 0.05 |
| $1.7x10^{11}$ PN/mL | 105 | 0.05 | 105 | 0.10 | 105 | 0.08 | 103 | 0.11 |

**Figure 2. AE-HPLC and PDGF-BB Expression Analysis of Lot B Subjected to Freeze-Thaw Stress**




*Temperature Stability*

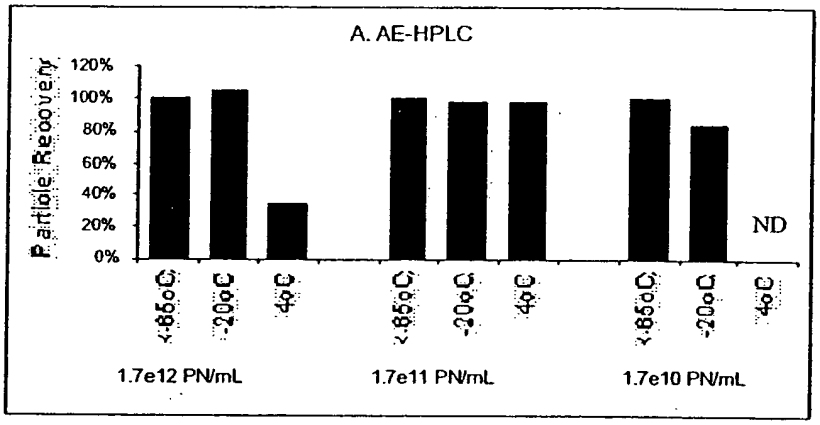
A six month temperature stability profile for Lot A was determined. Similar temperature stability studies for Lot B up to 3 months storage have been completed. Additional timepoints will be analyzed as the Lot B test articles become available. Test samples were stored at ≤ -65°C (control, data to which other test articles are compared), -20?C,

and 2-8°C. In addition, for Lot A, accelerated stability studies were performed at 35°C at the high and middle particle concentrations.

*Temperature Stability of Lot A*

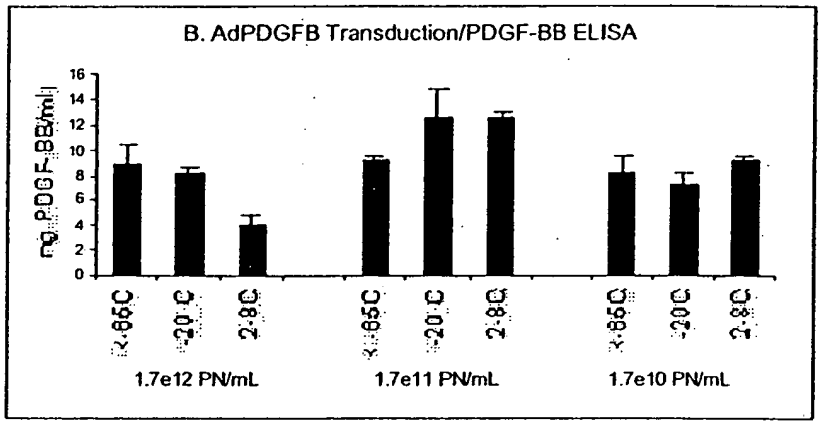
Lot A samples were analyzed at 2 weeks, 1 month, 3 month, and 6 month time points. The vector remained stable for at least 3 months, as determined by LLS, AE-HPLC analysis, and AdPDGF-B transduction/PDGF-BB ELISA. The 6-month stability data indicate all samples were stable at -20?C. Middle and low concentration samples were stable at 2-8?C. However, the high dose 2-8?C sample showed a >50% decrease in intact particle recovery and PDGF-BB expressed, as determined by AE-HPLC and AdPDGF-B transduction/PDGF-BB ELISA analyses (Figure 3 and Figure 4, respectively). Particle size remained at about 100 nm suggesting that no aggregation had occurred. The overall intensity of scattered light decreased ca. 40% in these samples, indicating loss of viral particles. The decrease of intact AdPDGF–B particles and PDGF-BB expression appears to be due to viral particle disintegration.

**Figure 3. Total Particle Recovery (AE-HPLC) of Lot A Stored for 6 Months**




ND- Not Determined

**Figure 4. AdPDGF-B Transduction/PDGF-BB ELISA Analysis of Lot A Stored for 6 Months**




A 2-week accelerated stability study was performed at 35°C. AE-HPLC and PDGF-BB ELISA were performed to determine the vector integrity and bioactivity, respectively. The middle-particle concentration, $1.7x10^{11}$ PN/ml, remained stable for 7 days, and showed a loss of particle recovery and a decrease in transgene expression at 14 days. At the higher particle concentration, $1.7x10^{12}$ PN/ml, there is a time dependent decrease in particle recovery and transgene expression beginning at day 1.

*Temperature Stability of Lot B*

Long-term temperature stability studies for Lot B are currently in progress. Analysis of LLS data demonstrated no changes in particle size or average size distribution for 3-month incubation at all temperatures. AE-HPLC indicated quantitative recovery of particles for all three concentrations for all three temperature conditions. The biological activity of the high concentration sample ($5.1x10^{11}$ PN/mL) was stable at 3 months. The biological activity of the middle concentration sample ($1.7x10^{11}$ PN/mL) could not be determined due to an artifactual low response of the positive reference (-65°C) control. Future stability determinations are planned for these samples in an ongoing study.

# EXHIBIT D

# Adenoviral Reference Material Working Group
# Bid Submission Form
# Vialing and Freezing Donation
# RFP 6.0

Item for Submission

Vialing of an Adenovirus 5 Wild-type reference material bulk provided in final formulation. Bulk should provide enough material to fill at 0.5 mL in 4500 to 5000 vials at 2 to 5 x $10^{11}$ particles/mL. Vials are intended to be stored at ≤-55?C until shipped under proper conditions to institution(s) responsible as repository. Bidder will include sterility test of vials as part of bid.

General Requirements for Bidding

Finishing activities must be done under documentation equivalent to CGMP. Institution will need to provide evidence of their experience in finishing purified adenovirus or other similar biologicals and detail their facility and its capabilities. The facility description should address procedures to ensure segregation during filling of the reference material.

The purified formulated adenovirus reference material will be supplied from other bid activities in this process along with minimum handling instructions.. Indicate your minimum requirements or concerns for acceptance of this material if not addressed by characterization called for in RFP 5.0. The bid should indicate the amount of time required from receipt of the purified, formulated bulk reference material until completion of vialing, freezing, and sterility test of final containers.

The bidder should arrange sterility testing of the vialed reference material.

The proposal should include details of the proposed method/container for shipping to ensure integrity of the vialed reference material upon arrival at the repository facility.

Documentation Requirements

Documentation should include detailed information on the proposed container/closure system(s). Information should also be provided on the maximum number of vials that can be filled at one time; the amount of time for filling the proposed number of vials; handling and storing of bulk before and during fill. The proposal should address the following issues:

?? Container/closure system's ability to provide stability for storage of adenovirus at ≤-55°C
?? If filling is to be staged in specific numbers of vials, information regarding the institution's limitations and how the institution proposes to address these limitations

?? If documentation and practices equivalent to CGMP are not utilized, what other type of data can be provided to support fill (e.g., media fill using the proposed container/closure)
?? Environmental monitoring program

It is expected that the Batch Records used for the filling procedure and the environmental monitoring data collected will be made available.

The proposal should contain the proposed method for sterility testing of the vialed reference material.

Proposed details related to shipping of adenovirus reference material vials to repository(ies).

Please complete the following fields:

*Contact Information – RFP 6.0*

| | |
|---|---|
| **Contact Individual:** | Joe Senesac |
| **Institution:** | Introgen Therapeutics, Inc. |
| **Address:** | 2250 Holcombe Blvd.<br>Houston, Texas 77030 |
| **Phone Number:** | (713) 610-4020 |
| **Email Address:** | j.senesac@introgen.com |

*Vialing and Freezing Donation Information – RFP 6.0*

Please indicate if your institution is also submitting proposals for the other activities:

☐ Donation of Cell Bank

☐ Donation of Ad5 Wild-type Virus

☒ Ad5 Wild-type Virus Bank Production

☒ Ad5 Wild-type Purified, Formulated Bulk Production

☐ Donation of Repository Services

☐ Donation of Supplies/Other Services

*Please attach:*
Procedure for Vialing
Proposed Container/Closure Information
Institution Capability Statement
Media Fill or Other Validation Information as available
Information on shipping

Submit this completed form and all attached information for receipt **by February 28, 2001** to the address below. Electronic submissions are encouraged. Final decisions will be communicated by or about March 31, 2001. Please note that all information submitted will be publicly available. Please do not mark any information confidential, as we cannot honor that request. Please estimate the cost and market value of donated goods and services.

**Williamsburg BioProcessing Foundation**
**Attn: Adenovirus Reference Material Working Group**
**4015 Killam Avenue**
**Norfolk, VA 23508**

**PH: 757-423-8823**
**FAX: 757-423-2065**

**EMAIL: advector@wilbio.com**

## A. Capability Statement

Introgen Therapeutics, Inc. (Company) is engaged in the manufacture, research and clinical development of viral vector-based gene therapies for cancer treatment. The Company utilizes a 12,000 square foot manufacturing facility at 2252 Holcombe Boulevard in Houston, TX for the production of viral vectors for non-clinical and clinical studies. This facility is fully commissioned and has been qualified via the production of three lots of an adenoviral vector, RPR/INGN 201, containing a functional copy of the human *p53* gene Ad5CMV-*p53*.

Introgen has produced over 30 clinical batches of various materials for Phase I to Phase III clinical studies. The facilities include class 100,000 down to Class 1,000 cleanrooms which provide two separate manufacturing suites and appropriate environments for Cell/Viral Culture, Purification, and Finishing activities. Key members of the manufacturing team have worked together at Introgen for greater than 5 years. Introgen also has fully staffed Quality Assurance and Quality Control departments for testing and oversight of production.

## B. Production Requirements

Material to be filled, accompanied by a completed Certificate of Analysis as defined in RFP 5.0. It is preferred that the material be provided in three aliquots if larger bulks are provided.

## C. Finishing and Freezing

Manual finishing operations will take place in a class 10,000 cleanroom equipped with a class 100 biosafety cabinet (BSC). All equipment and HEPA filters are in a current state of calibration or certification. The production will take place on a strict campaign basis, with the rooms thoroughly cleaned and disinfected prior to and at the end of production. Production personnel will be limited to working with a single construct each working day.

Product will be thawed at room temperature and sterile filtered into a sterile single use container before being held at refrigerated temperature overnight. The material will be held at room temperature during each fill. The fill line and fill needle are also sterile single use disposable. A programmable pump is used to accurately dispense the desired 0.5 mL fill volume. Crimping is performed with a West Capper crimper. A pilot study has been performed to demonstrate that an acceptable level of fill accuracy and consistency can be achieved.

Product will be filled in 3-4 fill runs (maximum 1500 vials per run), on separate days. A triplicate manual fill qualification of the appropriate size has been completed in a different room of the same class in this facility. A single fill qualification run of approximately 1500 vials will be executed in the room this product will be filled in. Standard environmental monitoring will be performed

for this operation, comparable to that done for Introgen fills of clinical-grade material.

The vials will be labeled using Introgen printed labels with a format agreed upon by the Adenoviral Reference Material Working Group. The label will contain the construct name, date of manufacture, a lot number and manufacturer. The label will not contain any concentration information, as that will be determined post-production.

## D. Production Schedule

The filling schedule will be set when the delivery date is known. The fill operation will require approximately 5-10 working days. Sterility testing will require two weeks for completion. Complete release testing will require approximately six weeks.

## E. Container Closure and Shipment

The glass vials used are contract supplied to Introgen Therapeutics, Inc. as sterile stoppered vials. The stoppered vials are aseptically packaged in stainless steel racks that are individually triple wrapped for cleanroom use. We have completed stability studies of up to 18 months in this container closure system.

Container closure details are as follows:

Vial - 3mL, 13MM Serum/Lyophilization Vial, Flint glass

Stopper – 13MM Grey Butyl Stopper

Crimp- Flip-off Button Crimp

Shipment of the vialed product will be on dry ice with the addition of a calibrated temperature trace device to document shipping conditions. The shipping container will be an EnduroTherm® insulated container.

## F. Certificate of Analysis and Specifications

See attached proposed Certificate of Analysis containing specifications for testing to be performed on each sub lot.

## G. Documentation

A detailed production report will be submitted upon completion of testing. The report will document the production methods, environmental monitoring results, and will include the completed Certificate of Analysis. This will be made available in lieu of completed production batch records.

**Introgen Therapeutics, Inc.**
**Certificate of Analysis for Adenovirus Serotype 5 Reference Material**
**Lot #**

Manufactured By: Introgen Therapeutics, Inc.
Adenovirus Vector Serotype 5 Reference Material
Virus Bank:
Cell Bank:

Lot Number
Date of Manufacture:
Number of Containers:
Store at -60°C or below

| Test | Sponsor | Specification | Result |
|---|---|---|---|
| Sterility USP & EP | MDS Panlabs | Sterile | |
| Bacterial Endotoxins Test | Introgen | Report Value | |
| Titration of Adenovirus Vector | Introgen | Report Value | |
| Virus Particle Enumeration by $A_{260}$ | Introgen | 2E11 – 5E11 vp/mL | |
| $A_{260}/A_{280}$ Ratio | Introgen | Report Value | |
| Purity by HPLC Ion Exchange | Introgen | Report Value | |
| Bovine Serum Albumin (ELISA) | Introgen | Report Value | |
| huDNA | TBD* | Report Value | |
| pH | Introgen | Report Value | |

I certify that the above information has been accurately transcribed.

By: _____ _____
Quality Assurance Representative Date

* Testing will be performed at a CGMP facility but Introgen has not yet determined which test site will be used.

# EXHIBIT E

## Award Recipients

At the March 22 meeting in Rockville, Maryland, the following project phases were awarded to organizations that had submitted proposals which fit the stated project requirements. The detailed acceptance criteria and award process are explained in the Meeting Minutes that have been posted on the website.

- Univ. of Alabama at Birmingham was awarded RFP-1: Donation of Cell Bank
- Canji, Inc. was awarded RFP-2: Donation of Viral (Starting) Material
- Canji, Inc. was awarded RFP-4: Production of Viral Bank
- Introgen, Inc. was awarded RFP-5: Production and Purification of Reference Material
- Introgen, Inc. was awarded RFP-6: Vialing and Freezing of Reference Material
- ATCC was awarded RFP-7: Repository Services

Award Recipients (By Recipient)-Characterization Phase-091701.xls
Award Recipients-Characterization Phase-091701.xls

RFP-3 pertains to materials and services that are being offered by various supply companies. No awards were granted for this RFP, as the entities working on the specific phases of the project will contact these suppliers to obtain the materials and services they need.

Return to WBF home

P.O. Box 1229, Virginia Beach, VA 23451-0229
Phone: (757) 423-8823 Fax: (757) 423-2065 E-mail: wbf@wilbio.com

VP, Product Development
Introgen Therapeutics, Inc.
(713) 797-9960
(713) 797-1349 (fax)

> -----Original Message-----
> From: klcarson@wilbio.com [mailto:klcarson@wilbio.com]
> Sent: Wednesday, April 11, 2001 2:50 PM
> To: s.gallagher@introgen.com
> Cc: Senesac, Joe; d.sublett@introgen.com; f_flagge@introgen.com;
> Aguilar-Cordova, Estuardo; Bauer, Steven; Hutchins, Beth; Simek, Stephanie
> Subject: Adenoviral Reference Material
>
> Shawn:
>
> I have been authorized by the Adenoviral Reference Material Working
> Group to formally advise your company that its donation bids have been
> selected for RFPs 5 and 6. These donation bids are:
>
> - RFP-5: Purified, Formulated Bulk Virus Production and Release
> Testing
> - RFP-6: Vialing and Freezing
>
> For your stages of the project, cells will be provided by Dr.
> Alexander Kotov at the University of Alabama at Birmingham, and the virus
> seed stock will be provided by Dr. Beth Hutchins at Canji, Inc.
>
> Once you have completed your work, you will be asked to ship the
> frozen vials to Dr. Charles Buck at ATCC.
>
> Various supply companies have offered to donate materials and
> services for this project. For existing donation offers, please go to our
> website at www.wilbio.com. I have also asked Frank Flagge and Dick
> Sublett to give me lists of the materials and testing services they will
> need (brand names and quantities), and I will make my best efforts to
> obtain much of what is needed from the many supply firms that attend our
> conferences.
>
> I am still working on a more detailed letter that I will send to you
> later today or tomorrow. Thank you for your participation in this
> project, and for your generous contributions. With your formal acceptance
> of this work, and your approval, we will give your company credit for its
> role in this project with an appropriate posting on our website.
>
> Keith
>
> Keith L. Carson
> Chairman
> Williamsburg BioProcessing Foundation
> PO Box 1229
> Virginia Beach, VA 23451
> (757) 423-8823
> FAX: 423-2065
> klcarson@wilbio.com
>

# EXHIBIT F

| | TILTER (PFU/ML) | VOL. (ml) | YEILD (PFU) | RECOVERY (%) | |
|---|---|---|---|---|---|
| | | | | STEP | ACC. |
| CUBE (LOW PERFUSION RATE KEEP GLUCOSE>1g/L) 1% TWEEN-20 BUFFER A | | | | | |
| HARVEST | | | | | |
| CLARIFICATION AND FILTRATION (0.22 UM) | | | | | |
| VIRUS SOLUTION | $2.6x10^9$ | 1900 | $4.9x10^{12}$ | | |
| CONC./DIAF. (10-FOLD CONC., DIAF INTO 1M NaCl BUFFER A | | | | | |
| CONC. SUP | $2.5x10^{10}$ | 200 | $5x10^{12}$ | 102% | |
| BENZONASE TREATMENT (O/N, RT, 100u/ml) | | | | | |
| TREATED SUP | | | | | |
| DILUTED WITH WATER TO CONDUCTIVITY= 22-25 mS/cm | $7x10^9$ | 700 | $4.9x10^{12}$ | 98% | 100% |
| DILUTED VIRUS SOLUTION | | | | | |
| | $1.5x10^{10}$ | 240 | $3.6x10^{12}$ | 73% | 73% |
| PURIFIED VIRUS | | | | | |
| CONC./DIAF (5-FOLD CONC) | $7x10^{10}$ | 50 | $3.5x10^{12}$ | 97% | 71% |
| FINAL PURIFIED PRODUCT | | | | | |

FIG. 23

# EXHIBIT G

ALTHEA technologies inc.

# QUANTIFICATION OF RESIDUAL HOST CELL DNA IN ADENOVIRUS PREPARATIONS

ABSTRACT: Four test articles were received for testing to determine the content of residual HEK 293 chromosomal DNA. Each sample was run using three assays that detect 120 bp, 411 bp, and 757 bp fragments of 18S rDNA. The DNA was quantitated using Real Time PCR and the ABI PRISM 7700 Sequence Detection System (TaqMan™ chemistry). Serial dilutions of HEK 293 chromosomal DNA were used as standards. The limit of quantitation and detection of each of the three assays is 1 pg of HEK 293 DNA per reaction.

Prepared For: Adenoviral Reference Material Working Group

Protocol: 7-PR-J116.001

Report No.: J116-001

INTROGEN
QUALITY CONTROL REVIEW
INITIALS BS DATE 24 Jan 02

| | | |
|---|---|---|
| Lead Operator: Christina Small | Signature: Christina Small | Date: 01.18.02 |
| Lab Manager: Holly Trotter | Signature: Holly Trotter | Date: 01.18.02 |
| Study Director: Chris Duffy | Signature: Christopher L. Duffy | Date: 01/18/02 |
| Quality Assurance: Michelle Hong | Signature: Michelle Hong | Date: 01/18/02 |

Contact: Chris Duffy
(858) 455-2197

# Table of Contents

## Study Information

| | |
|---|---|
| **Althea Protocol Number:** | 7-PR-J116.001 |
| **Test Article Identification:** | Adenoviral Reference Material<br>C/N 001503, P/N 10-00023<br><br>Adenoviral Reference Material<br>C/N 001504, P/N 10-00023<br><br>Adenoviral Reference Material<br>C/N 001505, P/N 10-00023<br><br>Adenoviral Reference Material<br>C/N 001506, P/N 10-00023 |
| **Objective:** | The objective of the study was to quantify residual HEK 293 DNA present in the test articles. |
| **Sponsor:** | Introgen Therapeutics, Inc.<br><br>2250 Holcombe Blvd.<br>Houston, TX 77030<br>Phone: 713-610-4089<br>Fax: 713-797-9913<br>Contact: Trish Landrum |
| **Testing Facility:** | Gene Quantification Laboratory<br>Althea Technologies, Inc.<br>3550 General Atomics Court, Bldg. 2<br>San Diego, CA 92121 |
| **Study Director:** | Chris Duffy<br>Phone: (858) 455-2197<br>Fax: (858) 455-2188 |
| **Schedule:** | |
| **Study Initiation:** | 11/08/01 |
| **Analysis Initiation:** | 12/17/01 |
| **Analysis Completion:** | 01/03/02 |
| **Study Completion:** | 01/18/02 |
| **Archives:** | Raw data, records, protocol, and a copy of the report will be maintained by the testing facility as described in Althea Standard Operating Procedure 3-QA-011, *Data Recording and Storage*. |

## Assay Description and Methods

### Assay Description

The Host Cell Quantitative PCR Assay uses TaqMan™ chemistry to estimate the amount of host cell DNA contained within adenoviral preparations via quantification of 18S rDNA, a high copy number gene. Three different PCR reactions produce overlapping amplicons of 120, 411 and 757 base pairs.

In addition, an independent plasmid system is used to confirm that the efficiencies of the PCR reactions were similar. 10 pg of the pAB plasmid is added to each sample and to a water control (pAB alone). A TaqMan™ PCR reaction is performed using primers and a probe homologous to unique sequences of the plasmid.

### Preparation of Standards

DNA was extracted from HEK 293 cells (ATCC AV-Cells – 1516 HEK 293 Lot#003553) as described in Althea Standard Operating Procedure (SOP) No. 3-GN-013, *Extraction of DNA from Whole Blood, Body Fluids and Cells Using Qiagen QIAamp® DNA Mini Kit.* The extracted DNA concentration was determined by UV absorbance as described in Althea SOP No. 3-EQ-011, *Operation and Maintenance of the Molecular Devices SPECTRAmax® Plus*$^{384}$ *Microplate Spectrophotometer.* Purified HEK 293 genomic DNA was digested for 1 hour at 37°C with *Apa* I (Promega Cat R636A, 10,000 units/ml). The DNA was purified using the Qiagen MinElute™ Reaction Cleanup Kit (CAT 28204). The concentration of the DNA was determined by UV absorbance as above and the DNA was ten-fold serially diluted from 10 ng/µL to 100 fg/µL.

### Preparation of Samples

Each test article was extracted in triplicate and the DNA was pooled prior to concentration determination. An in-house adenoviral precipitation method was used to purify DNA from the

sponsor provided samples as per Althea SOP No. 3-GN-013, *Extraction of DNA from Whole Blood, Body Fluids and Cells Using Qiagen QIAamp® DNA Mini Kit.*

**Real-Time Quantitative Polymerase Chain Reaction**

PCR amplification and fluorescence detection was performed using the ABI PRISM 7700 Sequence Detection System as described in Althea SOP No. 3-EQ-001, *Operation of the Perkin-Elmer 7700 (PE-7700) and Sequence Detection Software*, and Althea SOP No. 3-GN-005, *Quantitation of DNA Targets Using Universal Master Mix*. Three PCR reactions were performed on each test article DNA in two sets of quadruplicates using the oligonucleotide primers and fluorescent probe specific to 18S rDNA. An additional reaction was prepared with a known plasmid to check for the presence of PCR inhibitors. In addition to the test article reactions, each PCR run contained one set of standards and the PCR reagent control (NTC). Each of these controls was run in duplicate reactions.

Data were reported if all acceptance criteria described in the study protocol were satisfied. If a PCR run, or result for an individual test article failed to meet these criteria, repeat analyses were performed.

**Calculations and Statistical Methods**

For each PCR run, the Sequence Detection Software created a standard curve by plotting the quantitation assigned to each standard versus its mean threshold cycle. The software then performed a linear regression analysis to calculate the quantity (pg) of the target sequence in each reaction. The mean quantity of the duplicate reactions for each test article was calculated and reported.

Data generated by the Sequence Detection Software was exported into a Microsoft Excel spreadsheet that mathematically adjusts data from quantity of target per reaction to the quantity of target per microgram of DNA automatically.

## Results

**Table 1**

**Analysis Summary & Statistics**

| | Correlation Coefficient ($r^2$) | Slope | Threshold | Baseline | PCR Reagent Control ($C_T$) |
|---|---|---|---|---|---|
| 120 bp Amplicon | 0.998 | -3.490 | 0.05 | (1, 14) | 45, 40 |
| 411 bp Amplicon | 0.999 | -3.763 | 0.03 | (3, 15) | 45, 45 |
| 757 bp Amplicon | 1.000 | -3.910 | 0.04 | (3, 15) | 45, 45 |

**Table 1.** Analysis Summary & Statistics. A summary of data analysis parameters and resulting linear regression data is shown.

**Table 2**

**Residual Host Cell Quantification**

**pg HEK 293 DNA in 1µg Total DNA**

| | 120 bp Amplicon | 411 bp Amplicon | 757 bp Amplicon |
|---|---|---|---|
| Adenoviral Reference Material C/N 001503, P/N 10-00023 | <3 | <3 | <3 |
| Adenoviral Reference Material C/N 001504, P/N 10-00023 | <3 | <3 | <3 |
| Adenoviral Reference Material C/N 001505, P/N 10-00023 | <3 | <3 | <3 |
| Adenoviral Reference Material C/N 001506, P/N 10-00023 | <3 | <3 | <3 |

**Table 2. Residual Host Cell Quantification.** Results of real-time PCR quantification are shown for each aliquot of each test article.

**Table 3**

**Inhibition Test Results**

| | $C_T$ Value | Inhibition Present |
|---|---|---|
| Plasmid Alone | 17.80 | N/A |
| Adenoviral Reference Material C/N 001503, P/N 10-00023 | 17.65 | No |
| Adenoviral Reference Material C/N 001504, P/N 10-00023 | 17.55 | No |
| Adenoviral Reference Material C/N 001505, P/N 10-00023 | 17.50 | No |
| Adenoviral Reference Material C/N 001506, P/N 10-00023 | 17.44 | No |

**Table 3.** Inhibition Test Results. An exogenous plasmid was spiked into each sample and into water (Plasmid Alone). A sample with a $C_T$ value greater than that of the pAB Alone + 3 $C_T$ indicates PCR inhibition.

## Conclusions

A TaqMan™ based PCR assay was used to detect and quantify residual host cell DNA in adenoviral preparations. The data appearing in this report were derived from assays meeting the acceptance criteria specified in the study protocol. The quantitative real-time PCR analyses of adenoviral preparations were conducted in a GLP compliant manner.

Confidential

## Study Director's Approval

I accept responsibility for the conduct of this study that was performed in compliance with Title 21 of the U.S. Code of Federal Regulations, Part 58, *Good Laboratory Practices for Non-Clinical Laboratory Studies*.

Study Director

01/18/02
Date

Confidential

## Quality Assurance Statement

This study was performed in accordance with Good Laboratory Practices (GLP) for Nonclinical Laboratory Studies, Title 21 Code of Federal Regulations Part 58, and according to Protocol No. 7-PR-J116.001.

This report has been inspected and audited by the Quality Assurance Unit of Althea Technologies, Inc., and as far as can be reasonably established, the methods described and the results incorporated in the report accurately reflect the raw data produced during the study.

This study was subject to Quality Assurance Unit inspection(s) and/or audit(s) as follows:

| Inspection/Audit | Inspection/Audit Date | Date Reported to Management |
|---|---|---|
| GLP Audit | 11/08/01 | 11/08/01 |
| GLP Audit | 11/13/01 | 11/29/01 |
| Draft Report Audit | 01/18/02 | 01/18/02 |
| Final Report Audit | 01/18/02 | 01/18/02 |

I certify that this study report provides a true and complete record of the data generated.

Quality Assurance

1/18/02
Date